

REMARKS

Claim 8 is presently pending. Claim 8 has been amended to address the Examiner's concerns in Paper No. 12. Support for the amended claims language can be found throughout the specification but more particularly at the first sentence of the second paragraph of the Section 5.0 (at or around Page 6, line 21). Accordingly, the amendment is not deemed to constitute new matter.

No prior art rejections are presently of record.

I. The Present Claims Are Patentable And The Rejections of Record Should Be Withdrawn.

a) Rejections Under 35 U.S.C. § 101

The Examiner's rejections of Claim 8 under 35 U.S.C. section 101 is respectfully traversed. The Examiner has adopted the position that the claimed invention lacks a patentable utility due to its not being supported by either a specific and/or substantial utility or a well established utility.

The Examiner first rejects the invention of Claim 8 under 35 U.S.C. §101 because the specification does not disclose any gene that comprises the exon, and does not described the function of the gene. The Examiner's rejection is respectfully traversed. The Examiner is requested to consider that the described mutation was made using an insertional mutagen that disrupts the normal transcription and splicing of the gene encoding the wildtype form of the RNA binding protein (also annotated as the human ortholog of the couch potato gene). Unlike mutagenesis by gene targeting, insertional mutagenesis using gene trap vectors requires little to no prior knowledge of gene structure in order to produce the described mutated cell line. Accordingly, the Examiner's concerns about the specification containing no teaching of gene structure are largely irrelevant to the practice of the presently claimed invention. The presently described genetically engineered cell line was produced using a patented method and a patented vector that effectively "sends back" a reporter sequence (from a downstream endogenously encoded exon) that indicates which gene has been mutated by the integrated vector. This reporter sequence is the "tag" presented in the Sequence Listing (e.g., SEQ ID NO:328) and is broadly useful, *inter alia*, for a variety of bioinformatics uses as well as in the design of gene specific primers useful in subsequent PCR analysis. In this instance, those skilled in the art could have readily BLAST searched SEQ ID NO:328 against the public databases

to readily reveal the identity of the mutated gene. In fact, a similar analysis was performed prior to producing knockout mice from the described ES cell line.

The Examiner's concerns that the specification allegedly does not teach the function of the mutated gene basically makes the Applicants' point by highlighting the basic motivation for and fundamental usefulness of the present invention. If the function of the gene was in fact already known, what discovery-based motivation would there be to process the described ES cell line into corresponding knockout animals? The specification teaches a novel knockout allele of a gene having orthologs in a variety of model organisms and in humans. Unlike orthologous genes from non-mammalian model organisms, the described cells first enabled an analysis of the function of this gene in a physiological background that has proven to be an effective predictor of drug action in man. In this instance, the mutated ES cells were used as taught in the specification and as generally used in the field to show that the mutated gene plays a role in regulating circulatory homeostasis. Clearly, the described results are "useful" by any biomedical measure. As such, the Examiner is respectfully requested to withdraw the pending rejection of Claim 8 under 35 U.S.C. §101.

To the extent that the Examiner maintains the position that the described mutated ES cell line lacks a substantial utility, Applicants request that the Examiner consider the broader scientific acceptance of the inherent value of knockout mice in discovering the function of genomic sequence information. As evidence of such, the Applicants' respectfully direct the Examiner's attention to the winners of the 2001 Lasker awards. Reproduced below for the Examiner's convenience are the comments by Lasker award presenter Ira Herskowitz:

Albert Lasker Award for Basic Medical Research, 2001
Comments at the Awards ceremony
Presented by Ira Herskowitz

"The release of the human genome sequence in draft form makes this a landmark year in the history of biology. Now we know that we have 30,000 or so genes (or is it 50,000?). We are now faced with several important questions, which include:

First, what are the functions of these genes and the proteins that they code for? And, second, how can we use this information to improve human health?

Until the ability to knock out genes in the mouse was developed, determining the function of human genes seemed largely out of reach, tantalizingly so. For example, we might know of a human protein that is found only in certain cells of the brain and

suspect what it might do, but how can we find out? Or, we might know of a gene in the fruitfly that is necessary for its development and see that humans have a very similar gene. Does it perform a similar function in humans? A powerful way to link a gene to function is to study the behavior of a mutant that lacks that gene and then see what the mutant can and cannot do. It's somewhat like disabling an automobile by removing one part and then inferring the function of the part that was removed. But we can't knock out genes in a human, so how can such mutants be produced?

The mighty mouse has come to the rescue. Its genes are typically 95% identical in sequence to ours, and we share the vast majority of our genes with the mouse. Despite the obvious differences between human and mouse in morphology and in some physiological processes, these differences are greatly outweighed by our similarities: they have kidneys and brains like ours; they have an immune system and develop a lot like humans; and they get diseases such as cancer and others that affect their cardiovascular and nervous systems like us. In some respects, mice are "pocket-sized humans". The bottom line is that the mouse provides the opportunity, dreamed about for decades, to make the link between a mammalian gene and its function. How is this done?

Building on more than one hundred years of genetic and embryological studies of the mouse, Mario Capecchi, Martin Evans, and Oliver Smithies have created a magic wand by which it is possible to modify any mouse gene with exquisite precision -- to completely delete it or to produce a specifically altered form of the gene.

The same technology also makes it possible to go the other direction - instead of knocking out a mouse gene, it's possible to restore function to a gene that is defective.

Let's now look at the process by which a mouse knockout is constructed.

A key piece of starting material is a mouse gene that's already been cloned: it might be a mouse gene corresponding to a human gene or a mouse gene corresponding to a fruitfly or nematode gene. The goal is to construct a mouse that lacks this gene. The second key piece of starting material is a special mouse cell line where the gene is going to be knocked out.

There are three steps for constructing a mouse knockout. In the first, a cloned gene is manipulated in a test tube to delete all or part of the gene. This is routine molecular biology. In step two, the mutated DNA is introduced into special mouse cells, where the mutated DNA replaces a normal gene copy in the chromosome. The crucial aspect of this process is that the mutant gene has to find the related sequences in the chromosome, so-called homologous DNA sequences, and then undergo recombination to switch places with the good gene. The ability of the introduced DNA to find the homologous DNA sequences is called "gene targeting". There was no evidence for gene targeting in animal cells growing in culture and great doubt about whether this could be done. This is where Capecchi and Smithies made their most important contributions. In the third step, the cells with the targeted, inactivated gene are grown

into a mouse that has this inactivated gene. It was Martin Evans who isolated the cell lines that made this possible and showed that genetic changes introduced into these cells in culture could be transmitted through the germ line and into mutant, progeny mice.

Let's now look at our awardees.

Verona, Italy has given us not only Romeo and Juliet, but Mario Capecchi. His early days as a child included living in orphanages and on the street in war-torn Italy from 4-9 years of age, then growing up in a nurturing Quaker environment in Pennsylvania. I refer interested people to articles that are available on the Internet. Capecchi did his graduate work at Harvard with Jim Watson and was enormously productive, making textbook discoveries on molecular mechanisms underlying protein synthesis. This was a golden age of molecular biology. Mario learned his lessons well, and when he established his own laboratory at the University of Utah in 1973, he sought to bring molecular genetics to animal cells growing in culture and learn how to manipulate the genes of these cells. This led him to undertake a series of studies beginning in 1977 that demonstrated gene targeting in animal cells and culminated in the construction of one of the first knockout mice in 1989. His first indications of homologous recombination in animal cells were published in 1982 and fueled a series of logical and remarkable studies that provide the standard methods for knocking out mouse genes.

Oliver Smithies was trained as a biochemist, but throughout his scientific career, homologous recombination kept on cropping up, and he came to think about how it could be used to fix defective genes. Smithies was born in Halifax, England and raised in the United Kingdom. After studying at Oxford University, he came to the University of Wisconsin for postdoctoral studies in 1951 and was on the faculty there for 28 years, from 1960-1988. He is presently at the University of North Carolina at Chapel Hill, and may well have flown here in his own little plane to attend this luncheon. After important early contributions springing from his development of a method for fractionating proteins, he became intrigued by the structure and evolution of mammalian genes, which meant that he became involved in cloning these genes.

In the early 1980s, Smithies began to wonder whether homologous recombination - gene targeting - could be carried out experimentally to correct a defective gene, for example, a mutant globin gene. For this type of genetic correction to occur, exogenously introduced DNA would have to target to the homologous chromosomal DNA sequence and recombine with it. But was this possible? No one had demonstrated gene targeting in animal cells.

In 1985 Smithies and colleagues demonstrated that they could introduce a DNA segment containing part of the globin gene into cells and then find cells in which this DNA segment had targeted to the chromosomal globin gene. This was a tour-de-force of sophisticated molecular genetics. His strategy was completely different from that used by Capecchi and though laborious, the demonstration of targeting was unequivocal.

These studies from the Capecchi and Smithies laboratories provided one of the essential ingredients for constructing gene knockouts in mice, the ability to target genes in cultured animal cells. The crucial next step was to take mouse cell lines modified in this manner and produce mice from them.

The history of mammalian embryology is intellectually rich and filled with great practical applications. It was nurtured by the agricultural industry among others and involved important work with rabbits and mice. The UK can lay claim to many important contributions in this area, and thus Martin Evans is part of a distinguished tradition. Evans was born in the UK and graduated from Cambridge in 1963. He then went to University College London, where he studied vertebrate development using frogs. After working with a certain type of cancer cell line that could differentiate in cell culture and be used to generate whole mice, Evans set out to isolate normal cells from an early mouse embryo that would have similar properties. Work from Richard Gardner argued for the existence of such cells, but culturing them had been elusive. In 1981, Martin Evans and Matt Kaufman and, independently, Gail Martin, in the U.S. were successful in isolating such cells, which have become known as embryonic stem cells, "ES cells". Evans then carried out an important series of experiments with his students Allan Bradley and Elizabeth Robertson that demonstrated that these ES cells could contribute to the mouse germ line. They further showed that genetically manipulated ES cells could transfer their genetic changes to progeny mice. The importance of ES cells was immediately recognized by Capecchi and Smithies, who learned how to grow ES cells and demonstrated that they could carry out targeted genetic alterations with them.

The first knockout mice constructed by gene targeting were published in 1989, and the rest is history. More than 4,000 different knockout mice have been constructed in the last dozen years, and many more are in the works! To keep on top of this fast-moving field, I suggest looking at the Jackson Laboratory's website, where you can find columns called "It's a Knockout!" and "KO of the Month".

The ability to modify the genetic make-up of a mouse by design provides a wealth of information on the function of the gene that is knocked out. Every aspect of mammalian physiology is being penetratingly analyzed by this technique. Particularly notable are the discoveries made on how the immune system functions, which have enormous implications for human health. Knockout mice made it possible to demonstrate unequivocally the molecular basis for prion diseases such as mad-cow disease. Knockout technology is also used to create mice that have versions of human diseases such as cystic fibrosis, muscular dystrophy, atherosclerosis, and many others. These mice make it possible to follow the course of a disease and provide an opportunity to identify and test drugs to ameliorate or cure these diseases.

The ability to precisely tailor mouse genes has completely revolutionized the practice of biomedical science for the last decade and is likely to become even more important

in the decades to come. We are certain to reap an enormous bounty of information from knockout mice and reap great benefits for the improvement of human health.”

In view of the above remarks, there should be no doubt that the mutated ES cell clones of the present invention have a substantial, well established and credible utility.

In view of the fact that public and private efforts have spent several billion dollars to obtain human genomic sequence data (and that corporate partners have committed to spending millions of dollars for early access to human genomic sequence), one can fairly state that such genomic sequence data, in part or in whole, have a demonstrated, substantial, and specific utility fully within that contemplated by 35 U.S.C. section 101 (see also the issues of “Nature” (2001, 409:745-964) and “Science” (2001, 291:1304-1351 that were both dedicated Human genomic sequence data). The practical implementation of the present invention adds value to human genomic data by assigning critical functional annotation to the human sequence data. It is thus axiomatic that an invention that adds value to an asset having *demonstrated and substantial economic and scientific utility* must also have a substantial utility. As such, it should be clear that even in the broader context of the application as a whole (as opposed to the specifically claimed ES cell line), the presently described invention has a demonstrated substantial utility.

In the event that the Examiner may still have some lingering doubts, Applicants invite the Examiner to further consider the guidance of the National Institute of Health which issued a request for applications entitled TOOLS FOR INSERTIONAL MUTAGENESIS IN THE MOUSE on January 25, 2001 (RFA-DA-01-011 which stated in part:

“This RFA solicits proposals for development of tools and techniques for the establishment of random and targeted sequence-tagged insertion libraries of embryonic stem (ES) cells that can be used to generate mutant mice in which the expression of the tagged gene could be controlled temporally and spatially. The development of such a resource for wide distribution to the scientific community would make it possible to scan the sequence database for any gene of interest and order the corresponding targeted ES cell line. Ideally, the insertional mutagenesis system developed would permit a wide range of genetic analyses and manipulations, including enhancer-trapping, conditional knockouts, conditional expression or overexpression, etc. It also would permit the larger community of investigators to utilize genomic resources efficiently. This effort complements ongoing National Institutes of Health (NIH) efforts to create and characterize induced point mutations in mice using ethylnitrosourea (ENU) and provides a functional genomics tool to translate the information from the Mouse Genome

Sequencing Project. Further information about NIH initiatives on mouse genomics and genetics resources is available at <http://www.nih.gov/science/mouse>.”

Related to the above quote, the Examiner is respectfully invited to visit the website www.baygenomics.ucsf.edu which describes a publicly funded gene trapping effort that further supports Applicants’ position that the presently described invention has a well-established utility. As a point of technical comparison, the present application alone describes over 1,000 different ES cell clones that were generated using a technology that, in its present form, produces more identified ES cell clones in a month than the publicly funded BayGenomics effort has produced in a given year. In brief, that branch of the U.S. government that is specifically tasked with sponsoring technologies having high biomedical utility has already financially “validated” the utility of a related, albeit technically inferior, gene trap technology by providing many millions of dollars of funding for such efforts². In view of the direct governmental validation of biomedical utility of gene trapped murine ES cell clones, one cannot credibly assert that the presently described murine ES cell clones somehow lack a well-established utility.

The Examiner has also maintained the position that the described ES cells do not have a specific utility. In view of the fact that the Examiner has cited no prior art, singly or in combination, specifically enabling an alternative means of discovering the specific physiological role of the presently mutated gene, there can be little question that the described genetically engineered mutations provide an exquisitely specific resource for identifying the physiological role of the gene at issue. Simply put, the Examiner has not cited any specific teaching that could *hypothetically* enable the substantial, credible, and specific utility addressed by the presently described genetically engineered cells. Conversely, the described novel cells demonstrably mutate the specifically identified gene and clearly enable the discovery of the physiological/medical significance of this gene. In view of the functional characterization of the specific gene at issue, there can be no question that the described mutated cell line has an exquisitely specific utility.

The Examiner has apparently also taken issue with the “form” of the presently described cells because the described ES cells allegedly lack specific benefit in their currently available form. The

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It also bears mentioning that NIH guidelines stipulate that institutions receiving such funding commercialize inventions made using NIH funds.

Examiner is respectfully requested to consider that the described ES cells are akin to bioengineered seeds have long been held patentable. The same way that seeds are cultivated using routine methods to produce commercially valuable crops, routine methods that were well known to those skilled in the relevant art were used to “cultivate” the described ES cells into knockout animals as specifically contemplated and explicitly discussed in the specification as filed. Given that there can be no question of the well established utility of the resulting knockout animals, there can be no question that the enabling novel mutagenized ES cell line must also be patentable.

The Examiner is also respectfully requested to consider that novel genetically engineered cell lines have long been used by the academic community and Biotech industry to characterize biochemical pathways, in cancer screening, and to produce therapeutic products. A cursory review of issued U.S. patents conclusively shows that there can be no question that there is broad acceptance within the relevant scientific community that genetically engineered cell lines are useful in any of the above the applications described above, among others, have broad utility. The specifically exemplified novel cell line is useful for several of the above mentioned utilities (*e.g.*, it is known that ES cells will form tumors when introduced into syngeneic or nude mice, and cell lines having mutations in specific genes are patently useful for elucidating the biochemical pathways in which the products of those gene are involved, etc.), and that only a single utility, *in vitro* or *in vivo*, is sufficient to confer patentable utility. In brief, the Examiner’s reliance on *Brenner* is misplaced because those skilled in the art would clearly understand that the presently described novel cell line shares the broader utility already recognized by the Patent Office for a wide variety of patentable “research tool” cell lines. However, in the present case, the described cells provide the additional utility, virtually unique to this specific class of cells—the utility of being able to produce live animals containing a genetic complement largely derived from the mutated ES cells (note the recent patents broadly covering *unaltered* human ES cell lines). In brief, there can be no question that those skilled in the art would clearly recognized that the described ES cell line has a usefulness that transcends that of “a compound whose sole ‘utility’ consists of its potential role as an object of use-testing.” A quick review of issued U.S. patents indicates that the Patent Office has already determined that technologies directly relating to the generation or routine utilization of ES cells are broadly patentable, and that even aneuploid cell lines have a patentable utility. There is thus no rational basis in fact, or even policy, to somehow argue that a novel mutated ES cell line that

specifically enables the functional characterization of a specific gene somehow lacks a patentable utility.

In view of the overwhelming evidence of the substantial, credible, specific, and well-established utility of the presently claimed invention, the Applicants' respectfully request that the Examiner withdraw the pending rejection of Claim 8 under 35 U.S.C. section 101.

b) Rejections under 35 U.S.C. Section 112

The Examiner has also rejected Claim 8 under 35 U.S.C. section 112, first paragraph for alleged lack of written description for the claimed invention. The Examiner's rejection is respectfully traversed. The Examiner has correctly stated that the written description requirement generally requires that the specification convey to those skilled in the art that the applicant is effectively in possession of the claimed invention. Applicants respectfully invite the Examiner to reconsider this point given that, as specifically discussed in the specification, the described method of preparing the claimed ES cell clones requires the actual production and isolation of the described ES cell clones *prior to the generation of any sequence data*. The sequence reported in SEQ ID NO:328 represents exon sequence that clearly identifies the gene that has been mutated in the described ES cell line. Those skilled in the art would understand that actual possession of the described ES cell clone is a prerequisite of obtaining the exon sequence data reported in SEQ ID NO:328. As such, those skilled in the art would have no question that the Applicants are in actual possession of the described ES cell clones. In view of the above considerations, the Examiner is respectfully requested to withdraw the rejection of Claim 8 under 35 U.S.C. section 112, first paragraph for lack adequate written description.

Having said such, Applicants would further remind the Examiner that such issues of written description can routinely be dispensed with by the deposit of the described ES cell line. Although unnecessary given the teaching in the specification, Applicants will offer such a biological deposit if it remains the last impediment to issuance of Claim 8.

The Examiner's rejection of Claim 8 under 35 U.S.C. § 112, first paragraph over the alleged lack of utility/use of the claimed ES cells is respectfully traversed for the reasons provided at length in the Applicants' demonstration of the clear utility of the described invention.

The Examiner has also rejected Claim 8 under 35 U.S.C. section 112, second paragraph as allegedly indefinite over the use of the term "an exon sequence disclosed in SEQ ID NO:328". The

Applicants have amended Claim 8 to recite "the" exon sequence.... In so doing, the Applicants believe that they have avoided the ambiguity discussed by the Examiner. To the extent that the Examiner might prefer an alternative means of describing the invention, the Examiner is respectfully invited to contact the undersigned attorney and make such suggestion.

II. CONCLUSION

In view of the foregoing amendments and remarks, the Applicants believe that the application is in good and proper condition for allowance. Early notification to that effect is earnestly solicited.

If the Examiner feels that a telephone call would expedite the consideration of the application, the Examiner is invited to call the undersigned attorney at (281) 863-3333.

November 19, 2003
Date

Respectfully submitted,



Lance K. Ishimoto

Reg. No. 41,866

LEXICON GENETICS INCORPORATED
(281) 863-3333

Customer # 24231